XIII. "On the presence of Sulphocyanides in the Blood and Urine." By Arthur Leared, M.D., M.R.I.A., &c. Communicated by C. Handfield Jones, M.B. Cantab. Received June 17, 1869.

In the course of some investigations into the composition and uses of saliva, I was led to study one of its components, sulphocyanide of potassium, in relation to its presence and action in the system, in a way that has not hitherto been done.

Treviranus, in 1814, discovered that saliva became reddened by a persalt of iron in solution; and the reaction was afterwards stated by Tiedemann and Gmelin to be due to the presence of sulphocyanide of potassium*. A strange difference of opinion has nevertheless prevailed on the subject. Thus the reaction has been supposed to be caused by a taint of the saliva from carious teeth; whilst Bernard states that he found it took place only in the saliva of tobacco-smokers.

It is unnecessary to produce here the arguments on both sides of the question; the weight of authority is altogether in favour of the existence of the salt in saliva. By some of those, however, who have admitted that it is an ingredient of the secretion it has been regarded as a curiosity rather than as playing any part in the economy.

I have made numerous experiments for the purpose of satisfying myself as to the constancy with which a sulphocyanide exists in human saliva. For this end a solution containing twenty grains of perchloride of iron in an ounce of distilled water was employed. Such a solution is of a light-yellow colour, but it acts better than the paler solution of the persulphate of iron. The mode of procedure was very simple. The saliva to be tested was ejected on a surface of white porcelain, or upon a piece of white paper, and a drop of the test-solution added. The colour which the saliva assumed was compared with a scale of four shades of red on paper, resembling those produced by the sulphocyanide of iron. These shades were labelled "very marked," "marked," "faint," "a trace," and corresponded approximately with the colours struck by iron in solutions of sulphocyanide of potassium of the relative strength of $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$ of a grain to the ounce of distilled water.

Such a scale is appended herewith. An examination of the saliva of fifty persons taken consecutively, half being males and half females, and of ages ranging from under one year to 65 years, gave the following results:—

	Very marked.	Marked.	Faint.	A trace.	None.
25 Males	0	17	4	3	1
25 Females....	1	10	8	2	4

As regards the influence of age and other practical points, the numbers

* Recherches expérimentales, Physiologiques et Chimiques sur la Digestion. Paris, 1827.

are insufficient for the deduction of trustworthy results. Some points, however, which it is unnecessary to prove by tabulation, were well borne out, and these are sufficient for the present purpose.

It was established that in the saliva of the great majority of persons a red colour is struck with perchloride of iron.

It was ascertained that the existence of carious teeth is not requisite for the production of this reaction, because it occurred in many instances in which all the teeth were sound.

It was further ascertained that tobacco-smoking was not indispensable, because the colour was produced in many cases in which the individual never used tobacco.

It was also remarked that in all the cases in which the absence of the sulpho-cyanide was noted, although no definite disease was apparent, the health was feeble, and that, on the other hand, a marked reaction with iron usually corresponded with the ordinary indications of sound health. To this subject I shall afterwards have occasion to return. It is probable that, by means of evaporation, a sulphocyanide would in every instance have been detected in the saliva. But for practical purposes it is assumed that when not discovered by the means described, it is not present.

The particular base combined with the sulphocyanic acid in human saliva is a matter of little importance. It has lately been stated that it is not potassium, but sodium, which was long ago mentioned by Tiedemann and Gmelin as taking the place of potassium in the saliva of sheep.

The soluble sulphocyanide which exists in the saliva cannot be regarded as an excretion, because it passes with the saliva into the stomach. Whatever its use or its ultimate destination, it seemed probable that a salt of so stable a nature was not decomposed in its passage through the system. This suggested that I should look for it in the urine.

Iron, as is well known, yields a very characteristic test of the presence of sulphocyanides. One compound only which it forms, namely, that with meconic acid, is at all likely to be confounded with the sulphocyanide of iron. The great sensitiveness of this test also makes it peculiarly adapted for quantitative analysis, by means of colour.

I found in my first experiments that when the urine of a person in whose saliva a sulphocyanide was abundant, was concentrated by evaporation, a reddish-brown colour was caused by the addition of perchloride of iron. But owing to the dark colour assumed by the concentrated urine, and the mode in which precipitation occurred, no reliance could be placed on this as a proof of the presence of a sulphocyanide in the urine. The step which then suggested itself was to decolorize the urine by means of animal charcoal. But it turned out, when this was effected, that the colourless liquid gave no reaction with the persalt of iron. The following experiment was then tried:—

A solution of one grain of sulphocyanide of potassium in an ounce of

distilled water was filtered through animal charcoal. The filtrate was tested with the iron solution. There was no reaction whatever.

It was plain from this that animal charcoal possesses the power either of separating sulphocyanides from their solutions or of decomposing them.

Various other methods for separating the colouring-matter were now tried with more or less success. The most perfect of these, as regards the removal of colour, was the addition of a solution of sub-acetate of lead. But the use of this solution is open to the objection that acetate of iron, which is formed in testing for sulphocyanic acid, is itself red. It is true that the colour is not so intense as that which was actually formed in most cases; and it was possible in estimating the amount of the essential colouring-agent present by an easy application of the colour-test to deduct the amount of colour due to the acetate of iron.

A modification of the method employed by Professor Harley for separating the colouring-matter of the urine, for the purpose of obtaining urohæmatin, proved on the whole the best. It consists in evaporating the urine in a water-bath to the consistence of thick syrup, treating with alcohol, and adding gradually milk of lime. The filtrate from this mixture was found to be of a light-yellow colour, closely resembling that of the iron solution. The ferric solution was added to this filtrate so long as precipitation of oxide of iron occurred. The liquid now assumed a reddish colour, varying in depth according to circumstances. The mixture was then filtered; but it generally happened that, after standing some hours, a second filtration was necessary.

The coloured fluid obtained by either of these methods from evaporated urine is of a bright red colour, exactly resembling that formed by an aqueous solution of sulphocyanide of potassium with perchloride of iron.

In some respects the two solutions did not exactly agree.

The colour of an aqueous solution of sulphocyanide of iron is only affected by mineral acids when in considerable excess. But the colour formed with iron in evaporated urine is easily destroyed by these acids.

The colour of the pure solution is removed by perchloride of mercury, while that of the organic solution is not affected by the mercurial solution.

It is well known that in certain cases the presence of organic matter in solution greatly modifies chemical action. The action of acids in the present instance was a question of degree. The colour was removed from the urinary solution by a small quantity of a mineral acid, and it was removed or impaired in case of a pure solution by a greater quantity of acid.

The following observation throws light upon the action of perchloride of mercury in the respective solutions.

Perchloride of mercury at once destroys the colour of an aqueous solution of sulphocyanide of iron. But, as I have ascertained, if the solution has been previously boiled (and boiling was employed in the case of the

urinary solutions), the red colour is no longer destroyed by the mercurial solution.

Since, then, these difficulties are capable of removal, the argument by the method of exclusion in favour of the red colour being due to sulphocyanide of iron appears conclusive. There is in fact no other source from which the red colour could proceed in the process by which the urine was decolorized by milk of lime.

Some salt of sulphocyanic acid must, then, be admitted to be a component of the urine.

For the detection of the salt it is only necessary to evaporate eight ounces of urine in a water-bath. If milk of lime be employed as the decolorizing agent which, for reasons already stated, is to be preferred, the urine should be concentrated to a thick syrup.

In the present stage of my inquiries many details are purposely omitted, particularly those which refer to the quantitative determination of the sulphocyanide in many different samples of urine. I may mention, however, that I found the average quantity present in healthy urine to amount to about $\frac{1}{8}$ of a grain in sixteen ounces.

Since, then, a sylphocyanide was found in the urine, and was previously known to exist in saliva, it was natural to look for it in other secretions. It was therefore sought for in a large quantity of cow's milk, and in two ounces of human sweat, but with negative results.

Two ounces of pure pus from a cyst on a man's back were also examined, but no sulphocyanide was found.

But as sulphocyanic acid was proved to exist in a secretion from which it may be presumed to enter the blood, and also in an excretion derived from the blood, it was to be expected that it would be found in the blood itself.

The blood operated on was in every instance diluted with an equal part of distilled water. The mixture was then evaporated in a water-bath until the red colour was altogether lost, and brown coagula, with apparently little fluid, remained. The mass was strained through muslin by pressure of the fingers. The filtrate was then decolorized by one of the processes already described. Briefly stated, then, it was found that a sulpho-cyanide exists in the blood of man, and in that of the pig, fowl, turbot, salmon, and toad.

I also found that when the serum of pig's blood, procured as free from colour as possible and diluted with an equal portion of water, to prevent complete coagulation, was treated with a solution of perchloride of iron, it became red in a marked degree. This result has a special interest, because it was obtained without any previous chemical manipulation, and the presence of a sulphocyanide was thereby proved. And this curious circumstance was also ascertained. If a few drops of a weak solution of sulphocyanide of potassium be mixed with this reddened and diluted serum, and the iron solution is again added, no increase of colour is produced. This

shows that while the sulphocyanide naturally present in the serum is capable of combining with added iron, the serum possesses the power of preventing the formation of sulphocyanide of iron when both compounds are added and intermixed with it.

An analogous masking of chemical action is described by Bernard. He found that when a solution of lactate of iron is mixed with serum, and a solution of cyanide of potassium is then added, prussian blue is not formed, as would be the case if the solutions were mixed in water instead of serum.

I have not been able to decide positively whether the sulphocyanide is or is not confined to the serum. Analysis, after combustion, is unsuitable, because sulphocyanides are formed in the combustion of organic matter. But so far as I have been able to determine from the maceration of the clot in water, the sulphocyanides exist only in the serum.

The foregoing facts point either to the presence of free sulphocyanic acid, or of sulphocyanide of potassium, or sodium, or of both, in the serum of the blood. This leads to the consideration of that much-vexed question, the cause of the red colour of the blood. So far as concerns exact colour, nothing more closely resembles blood than a solution of sulphocyanide of iron. This is *primâ facie* evidence that red blood owes its colour to the iron compound. The iron is known to be localized in the globules. These are surrounded by a fluid containing sulphocyanic acid in a combination which easily yields the acid when required. Such is the theory at present suggested.

I am not unaware of the difficulties in the way of its acceptance. The colour of hæmatin cannot, it is said, depend upon the iron it contains, because nearly the whole of the iron may be removed without affecting the colour of the hæmatin*. But it is not stated that all the iron is ever removed, and it may be that a very small proportion suffices for the formation of the colour, while the larger proportion of the metal is held in reserve in the globules in the same manner as sulphocyanic acid appears to be in the serum.

Having found a sulphocyanide in the blood, it next occurred to me to look for it in the eggs of birds. It is natural to suppose that, since in the process of incubation red blood is formed, its assumed elements of colour would be found in the egg before incubation. This supposition proved correct. Fortunately the albumen of the hen's egg affords a ready means of research. It is only necessary to mix it with an equal quantity of distilled water, by which complete coagulation by the iron solution is prevented. The albumen of a hen's egg weighs about 300 grains, and this quantity was found to contain $\frac{1}{20}$ of a grain of sulphocyanic acid. The yolk was intimately mixed with water, then evaporated to dryness in the water-bath, and extracted with alcohol; but no trace of the salt could be detected. It is probable, therefore, that the sulphocyanide exists exclusively in the

* Elements of Chemistry. By W. Miller, M.D., 3rd edit. p. 872.

albumen, which, as the process of vivification proceeds, enters into combination with iron, which originally exists in the yolk.

The presence of a sulphocyanide in saliva must be referred to one of two sources. It is either an exclusive product of the secretion itself, or it previously exists in the blood and is extracted from it as a component of the saliva. The amount of sulphocyanide found in different analyses varies greatly; my own results show only about half a grain in twenty ounces of saliva from a healthy subject. This nearly agrees with the observations of Bidder and Schmidt. Wright makes the quantity very much greater. If we take the estimate at only half a grain in twenty ounces of saliva, and reckon this to be the quantity of the secretion swallowed in twenty-four hours, the salt might be probably found in the blood and in the urine.

If, however, my experiments have been rightly interpreted, it is certain that sulphocyanide of potassium or sodium is not a mere product of the salivary glands, We have seen that it is found in the blood of all orders of vertebrate animals, and we know that fish do not possess salivary organs. Assuming that it is extracted out of the blood, what is its use in the saliva?

Considering its composition, it seemed possible that it acted either as an antiseptic or else as an agent which prevented fermentation in the alimentary canal, and thus indirectly aided digestion.

The conditions which favour the fermentation of saccharine matter, namely, acidity and the proper temperature, are constantly present in the stomach. Is sulphocyanide of potassium in saliva destined to check this fermentation, which, under favourable circumstances, may occur in less than an hour?

Carefully conducted experiments proved that it neither possesses the power of preventing ordinary fermentation nor that of checking it when already in action.

We shall now see what is its action in preventing putrefaction. Two equal portions of roast mutton were placed, the one in water, and the other in the same quantity of a solution of sulphocyanide of potassium of the strength of 1 grain of the salt to 1 ounce of water. After some weeks the meat which had lain in water was found to be broken up into shreds, and was quite putrid; that in the sulphocyanide solution was merely softened, and had a sour smell, but was not putrid.

Sulphocyanide of potassium, therefore, possesses an antiseptic power; but whether or not this property comes into operation in the alimentary canal is a question I cannot now decide.

I have made many quantitative analyses to determine the amount of sulphocyanide eliminated with the urine in various diseases, including typhus, typhoid, and scarlet fever.

Not to enter into details at present, it will be sufficient to state what the results showed with much uniformity. In all diseases in which wasting of the body was marked, the excretion in the urine of a sulpho-

cyanide, in common with some other substances, was unusually great. This increase of it in the urine was found to correspond with its decrease in, or more frequently its disappearance from, the saliva. This circumstance goes to prove that the salt is not formed by the saliva, but is an ingredient of the blood itself.

XIV. "On some Elementary Principles in Animal Mechanics."—No. II. By the Rev. Samuel Haughton, M.D. Dublin, D.C.L. Oxon., Fellow of Trinity College, Dublin. Received June 14, 1869.

In a former communication to the Royal Society on this subject (Proceedings, 20th June 1867), I endeavoured to establish the two following principles:—

I. *That the force of a muscle is proportional to the area of its cross section.*

II. *That the force of a muscle is proportional to the cross section of the tendon that conveys its influence to a distant point.*

The first of these principles is true under all circumstances, but the second requires to be modified somewhat in its statement. If the conditions as to friction of the tendons that convey the action of the muscles to a distant point be the same, then the force of the muscles will be proportional to the cross sections of the tendons; but if the tendons be subjected to different amounts of friction, then the areas of their cross sections will cease to be proportional to the forces of the muscles, as represented by the areas of their cross sections.

In my former paper (No. I.), I selected, in illustration of principle II., the long flexor tendons of the toes of the Rhea and other struthious birds, and showed that the cross sections of the muscles and tendons bore, approximately, a constant ratio to each other. Now, in the *Struthionidæ*, the conditions as to friction of the long flexor tendons of the toes are similar although different in each species, and hence it was easy to prove that the ratios of the cross sections of the muscles and tendons were nearly constant.

When, however, muscles and tendons, variously conditioned as to friction, are compared together, the constancy of the ratio of their cross sections disappears, and undergoes a variation depending on the friction to which both muscles and tendons are exposed.

In order to ascertain the proportion of the cross section (or force) of a muscle to the cross section (or strength) of its tendon in the human subject, I made the following observations on the right arm and hand of a well-developed male subject in the Royal College of Surgeons in Ireland, in March 1868.

I first ascertained the specific gravities of the muscles and tendons, with the following results:—